REMARKS

Preliminary Remarks

Reconsideration and allowance of the present application based on the following remarks are respectfully requested. Claims 1-20 are currently pending in this application. Claims 1-8, 17, 19, and 20 are withdrawn from consideration as being directed to a non-elected invention. Claims 9-16 and 18 remain at issue.

In paragraph 1 of the official action, the examiner acknowledged receipt of German Patent Application Nos. 10043336.7 and 10126422.4 filed on September 2, 2000 and May 31, 2001. Nevertheless, the examiner asserted the applications are in German and cannot be used to establish an earlier effective filing date for the claimed subject matter. In response, the applicants submit that they shall submit, under separate cover, certified English translations of German Patent Application Nos. 10043336.7 and 10126422.4.

In paragraph 6 of the official action, the examiner objected to the title for allegedly lacking description. The examiner suggested the title "Methods of Making L-Amino Acids in Coryneform Bacteria Using the sigE Gene." The applicants have adopted this title and are grateful for the examiner's suggestion.

In paragraph 7 of the official action, the examiner objected to the abstract under MPEP §608.01(b) as lacking sufficient description of the disclosed subject matter. The applicants have amended the abstract to include the source species of the sigE gene, *Corynebacterium glutamicum*.

In paragraph 8 of the official action, the examiner objected to the specification for lacking continuity data in the first paragraph. The applicants have amended the specification on page 1 to include the appropriate benefit information at this time and hereby request withdrawal of the objection.

In paragraph 9 of the official action, the examiner objected to the order of the specification's content. Specifically, the examiner alleged that the "Brief Description of The Drawings" at the end of the specification should be moved to page 4 before the "Detailed Description of the Invention." The applicants have amended the specification to arrange the "Brief Description of the Drawings" before the "Detailed Description of the Invention."

In paragraph 10 of the official action, the examiner objected to use of the phrase "enzyme sigma factor E" as it is allegedly confusing. At page 6, line 20, the applicants have amended the phrase to now read "RNA polymerase sigma-E factor."

In paragraph 11 of the official action, the examiner objected to claims 15 and 16 for having an improper structure of a Markush group. By the foregoing amendment, the applicants have amended the previously presented Markush group.

In paragraphs 12 and 13 of the official action, the examiner objected to claim 18 for allegedly being directed to a non-elected subject matter by virtue of its dependence on "one or more of the preceding claims," which includes non-elected Claims 1-8 and 17. In addition, the examiner alleged claim 18 does not further limit claims 9-16 because each of these claims are directed to fermentation of coryneform bacteria, which is a synonym of the genus *Corynebacterium*. Claim 18 is now dependent upon claim 9, which is part of the elected claimed invention.

New claim 21 is directed to a process according to claim 9, wherein said L-amino acid is L-lysine. New claim 22 is directed to the process according to claim 9, wherein said nucleotide sequence comprises nucleotides 302 to 949 of SEQ ID NO: 1. Support for new claims 21 and 22 can be found throughout the specification, for example, page 5, line 13-22, page 5, line 31 to page 6, line 6, page 20, line 4-8, and originally filed claim 11.

New claim 23 is directed to a process for producing L-amino acids comprising transforming a coryneform bacterium with a vector which includes a *sig*E gene having the polynucleotide sequence of SEQ ID NO: 1, wherein said *sig*E gene is under control of a promoter which allows the overexpression of said *sig*E gene, culturing said bacterium in a medium suitable for expression of the *sig*E gene to thereby produce L-amino acids, and isolating the L-amino acids. Support for new claim 23 can be found throughout the specification, for example, in originally filed claims 9 and Examples 2 and 4.

New claim 24 is directed to a method for the preparation of L-amino acids comprising culturing coryneform bacteria, which include an overexpressed *sig*E gene having a polynucleotide sequence which encodes the amino acid sequence of SEQ ID NO: 2, in a medium suitable for the expression of the *sig*E gene to thereby produce L-amino acids. Support for new claim 24 can be found throughout the specification, for example, originally filed claim 9 and Examples 2 and 5.

New claims 25-30 are directed method according to 24 further comprising isolating L-amino acids using coryneform bacteria like *C. glutamicum*, transformed with a plasmid vector comprising the nucleotide sequence of SEQ ID NO: 1. Support for new claims 25-30 can be found throughout the specification.

The applicants do not intend by these or any amendments to abandon subject matter of the claims as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

Patentability Remarks

Rejection Under 35 U.S.C. §112, Second Paragraph

In paragraphs 14-20 of the official action, the examiner rejected claims 9-16 and 18 under 35 U.S.C. §112, second paragraph, as being indefinite. With regard to claims 9-16 and 18, the examiner alleged the metes and bound of the phrase "the *sig*E gene of nucleotide sequences which code for it" is unclear. The examiner further asserted the claim appears to claim more than one gene other than the *sig*E gene of *C. glutamicum* (SEQ ID NO: 1 encoding SEQ ID NO: 2). The examiner suggested drawing the claim to using enhanced SEQ ID NO: 1 or any DNA encoding SEQ ID NO: 2 to clarify the metes and bounds of the claimed invention. The examiner further alleged the *sig*E gene that is enhanced must be endogenous *Corynebacterium* sigE gene of the cell claimed or is the overexression from some particular species of *Corynebacterium*, like *C. glutaicum* or *C. melassecola*. Additionally, the examiner asserted the phrase "nucleotide sequence which codes for it" is unclear. The examiner also asserted the phrase "in particular overexpressed" in claims 9 and 13 is unclear. The examiner alleged that the terms "biosynthesis pathway" and "metabolic pathways" in claims 10, 11 and 18 are unclear because the skilled artisan would be unable to identify enhancement or reduction of additional genes read into the claims. With regard to claims 14 and 18, the examiner further alleged that the phrase "regulatory properties of the polypeptide (enzyme protein) for which the polynucleotides *sig*E codes" is unclear because no regulatory properties are mentioned in the specification or the art. With regard to claims 15, 16, and 18, the examiner asserted the structure "15.1, 15.2" etc. is confusing because similar numbering is used for the claims. In addition, the examiner alleged the article "the" in front of the genes of claims 15 and 16 was indefinite because the identification of which gene is unclear. Finally, with regard to claims 15 and 18, the examiner alleged the phrase

"codes for lysine export" was unclear because genes do not code for functions, but rather code for enzymes or proteins that have function.

Solely for the purpose of expediting prosecution, and without prejudice to the applicants' right to seek broader claims in a continuing application, the applicants have canceled claims 10, 11, and 14 without prejudice, thereby obviating the rejection of these claims (*i.e.*, terms for "biosynthesis pathway," "metabolic pathways," and "regulatory properties of the polypeptide (enzyme protein) for which the polynucleotides sigE codes").

Amended claim 9 is directed to a process for the production of an L-amino acid comprising (a) culturing a coryneform bacterium under conditions suitable for overexpression of the *sig*E gene having the nucleic acid sequence as set forth in SEQ ID NO: 1 and encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 2 (b) enriching the medium or the cells of the bacterium and (c) isolating the L-amino acid. Support for amended claim 9 can be found throughout the specification, for example, in originally filed claim 9 and Example 2.

The examiner has acknowledged that the metes and bounds of claim 9 would be clear if the claims were directed to using enhanced SEQ ID NO: 1. In addition, the phrase "in particular overexpressed" has been removed from claims 9 and 13 thereby rendering this rejection moot.

Amended claim 15 is directed to the process according to claim 9 wherein in a *C. glutamicum* strain, one or more of the genes selected from the following group is overexpressed: (a)the dapA gene which codes for dihydrodipicolinate synthase, (b)the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase, (c) the tpi gene which codes for triose phosphate isomerase, (d) the pgk gene which codes for 3-phosphoglycerate kinase, (e) the zwf gene which codes for glucose 6-phosphate dehydrogenase, (f) the pyc gene which codes for pyruvate carboxylase, (g) the mqo gene which codes for malate-quinone oxidoreductase, (h) the lysC gene which codes for aspartate kinase, (i) the lysE gene coding for a protein for lysine export, (j) the hom gene which codes for homoserine dehydrogenase, (j) the ilvA gene which codes for threonine dehydratase, (k) the ilvBN gene which codes for acetohydroxy-acid synthase, (l) the ilvD gene which codes for dihydroxy-acid dehydratase, and (m) the zwa1 gene which codes for the Zwa1 protein.

Support for amended claim 15 can be found throughout the specification, for example, originally filed claim 15 and pages 12 and 13 of the specification.

Amended claim 16 is directed to the process as claimed in claim 9, wherein in a *C. glutamicum* strain, one or more of the genes selected from the following group is deleted: (a) the pck gene which codes for phosphoenol pyruvate carboxykinase, (b) the pgi gene which codes for glucose 6-phosphate isomerase, (c) the poxB gene which codes for pyruvate oxidase, and (d) the zwa2 gene which codes for the Zwa2 protein. Support for amended claim 16 can be found throughout the specification, for example, originally filed claim 16 and page 14 of the specification.

Both claims 15 and 16 have been amended for clarity purposes. Also, claims 15 and 16 state the overexpression of *sig*E can be combined with either the overexpression or deletion of the above-mentioned listed genes of *C. glutamicum*. Finally, the applicants have adopted the examiner's suggestion for replacing the phrase "codes for lysine export" in claim 15 for which the applicants are grateful.

In view of the foregoing amendments and remarks, the applicants respectfully submit the rejection of claims 9-16, and 18 under 35 U.S.C. §112, second paragraph, has been overcome and should be withdrawn.

Rejections Under 35 U.S.C. §112, First Paragraph, Written Description

In paragraph 21 of the official action, the examiner rejected claims 9-16 and 18 under 35 U.S.C. §112, first paragraph, for containing subject matter which was not described in the specification. Specifically, the examiner alleged the *sig*E gene is described as encoding "an enzyme sigma factor E with no further explanation of this enzymatic activity. The examiner acknowledged a single example of a *sig*E protein is described in SEQ ID NO: 2, but no indication of how this structure is related to the noted function. The examiner concluded one of skill in the art would be unable to predict the structure or function of other members of this genus by virtue of the instant disclosure and are thus not adequately described.

As stated above, amended claim 9 is now directed to a process for the production of an L-amino acid comprising (a) culturing a coryneform bacterium under conditions suitable for overexpression of the *sig*E gene having the nucleic acid sequence as set forth in SEQ ID NO: 1 and encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO:

2 (b) enriching the medium or the cells of the bacterium and (c) isolating the L-amino acid. The applicants submit claim 9 is no longer directed to an enzyme sigma factor E without further explanation of the enzymatic activity. Rather, claim 9 is now directed to a coryneform bacterium that can overrexpress the *sig*E gene which encodes for the RNA polymerase sigma E factor and assist in transcription of genes.

Claims 12, 13, 15, 16, and 18 are ultimately dependent upon claim 9 and therefore are also drawn to a particular *sig*E gene sequence thereby obviating the rejection directed to these claims. In view of the foregoing amendments and remarks, the applicants respectfully submit the rejection of 9-16, and 18 under 35 U.S.C. §112, first paragraph, have been overcome and should be withdrawn.

Rejection Under 35 U.S.C. §112, First Paragraph, Enablement

Claims 9-16 and 18

In paragraph 22 of the official action, the examiner rejected claims 9-16 and 18 under 35 U.S.C. §112, first paragraph, for lack of enablement. Specifically, the examiner alleged that while the specification is enabling for methods using coryneform bacteria with overexpressed sigE and/or amino acid biosynthetic genes and with deleted amino acid reduction genes, does not reasonably provide enablement for methods using bacteria with enhancement and/or elimination of pathways or attenuation of such genes. The examine further asserted that while the specification enables overexpression of the sigE gene (overexpression including means of art such as strong promoter and increased copy number), it does not enable enhancement of said gene.

As stated above, amended claim 9 is directed to a process for the production of an L-amino acid by culturing coryneform bacteria under conditions suitable for overrexpression of the sigE gene having the nucleic acid sequence as set forth in SEQ ID NO: 1. The examiner has acknowledged that the specification enabled overexpression of the sigE gene (see official action, page 11). The aspect of enhancing the sigE gene is no longer an aspect of the claimed invention.

As discussed above as well, amended claim 16 is directed to the process as claimed in claim 9, wherein in a *C. glutamicum* strain, one or more of the genes selected from the following group is deleted: (a) the pck gene which codes for phosphoenol pyruvate

carboxykinase, (b) the pgi gene which codes for glucose 6-phosphate isomerase, (c) the poxB gene which codes for pyruvate oxidase, and (d) the zwa2 gene which codes for the Zwa2 protein. The examiner has acknowledged that the specification enables methods that delete said pathways so that the pathways are "switched off." In view of the foregoing amendment and remarks, the applicants respectfully submits the rejection of claims 9-16 and 18 under 35 U.S.C. §112, first paragraph, has been overcome and request withdrawal of the rejection.

Claim 14

In paragraph 23 of the official action, the examiner rejected claim 14 under 35 U.S.C. §112, first paragraph, for allegedly failing to comply with the enablement requirement. Specifically, the examiner alleged that raising the regulatory properties of a sigE polypeptide for use in the claimed methods would require undue experimentation. The examiner further alleged that not only are the regulatory pathways of the sigE polypeptide not described in the specification or known in the art, but also the normal activity of sigE polypeptides is not described. The examiner concluded that without any indication of function, it is wholly unpredictable how a skilled artisan would be able to regulate such an activity and therefore the claim is not enabled.

As discussed above, claim 14 has been canceled without prejudice hereby rendering the rejection of this claim under 35 U.S.C. §112, first paragraph, moot. In view of the foregoing amendment, the applicants respectfully request withdrawal of this rejection.

Claim 15

In paragraph 24 of the official action, the examiner rejected claim 15 under 35 U.S.C. §112, first paragraph, for allegedly failing to comply with the enablement requirement. Specifically, the examiner alleged that while the specification was enabling for methods using known feedback-resistance aspartate kinase and threonine dehydratase, the specification does not reasonably provide methods using unknown feedback-resistance aspartate kinase and threonine dehydratase. The examiner asserted identifying novel feedback-resistance aspartate kinase and/or threonine dehydratase polypeptides for use in the claimed methods would require undue experimentation.

As discussed above, amended claim 15 is directed to the process according to claim 9 wherein in a *C. glutamicum* strain, one or more of the genes selected from the group is

overexpressed: (a)the dapA gene which codes for dihydrodipicolinate synthase, (b)the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase, (c) the tpi gene which codes for triose phosphate isomerase, (d) the pgk gene which codes for 3-phosphoglycerate kinase, (e) the zwf gene which codes for glucose 6-phosphate dehydrogenase, (f) the pyc gene which codes for pyruvate carboxylase, (g) the mqo gene which codes for malate-quinone oxidoreductase, (h) the lysC gene which codes for aspartate kinase, (i) the lysE gene coding for a protein for lysine export, (j) the hom gene which codes for homoserine dehydrogenase, (j) the ilvA gene which codes for threonine dehydratase, (k) the ilvBN gene which codes for acetohydroxy-acid synthase, (l) the ilvD gene which codes for dihydroxy-acid dehydratase, and (m) the zwal gene which codes for the Zwal protein. The applicants respectfully submit that the specification provides guidance to the two genes encoding for the threonine dehydratase. The first gene is ilvA isolated and described in Mockel *et al., J. of Bact.* 8065-8072 (1992). The second gene is ilvA(Fbr), which is an allele that encodes for a "feed back resistant" threonine dehydratase (see also Mockel *et al., Molecular Microbiology* 13:833-842 (1994)). Accordingly, the applicants believe claim 15 is directed to a finite number of feedback-resistant threonine dehydratases as discussed on page 13, lines 14-18 of the specification, and thereby provides sufficient guidance to one of skill to use the claimed method.

In view of the foregoing amendments and remarks, the applicants respectfully submit the rejection of claims 9-16 and 18 under 35 U.S.C. §112, first paragraph, for lack of enablement, has been overcome and should be withdrawn.

Rejection Under 35 U.S.C. §102(b), Anticipation

In paragraph 25 of the official action, the examiner rejected claims 9, 12, 13, and 18 under 35 U.S.C. §102(b) as allegedly being anticipated by Kimura *et al.* (EP 0864654) (hereafter Kamura *et al.*). Specifically, the examiner alleged Kimura *et al.* teach methods of making amino acids by overexpression of a gene encoding a sigma factor, using plasmids in the methods, coryneform as microorganisms for the disclosed methods, and the collection of amino acids by known methods.

In view of the foregoing amendments, the applicants respecfully submit Kamura *et al.* does not anticipate the process of claim 9. Specifically, Kamura *et al.* does not disclose the process of fermenting L-amino acids in coryneform bacteria using the *sig*E gene nucleotide

sequence SEQ ID NO: 1 isolated from *Corynebacterium glutamicum*. In contrast to applicants teachings, Kamura *et al.* discloses using the *E. coli sig*E gene sequence in conjunction with the induction of heat shock proteins to increase the productivity of fermentation products. Nothing in Kamura *et al.* is mentioned regarding the *sig*E nucleotide sequence from *Corynebacterium glutamicum*.

The anticipation by Kamura *et al.* should not be extended to new claims 23 or 24 as well. New claim 23 is directed to a process for producing L-amino acids comprising (a) transforming a coryneform bacterium with a vector which includes a *sig*E gene having the polynucleotide sequence of SEQ ID NO: 1, wherein said *sig*E gene is under control of a promoter which allows the overexpression of said *sig*E gene, (b) culturing said bacterium in a medium suitable for expression of the *sig*E gene to thereby produce L-amino acids, and (c) isolating the L-amino acids. New claim 24 is directed to a method for the preparation of L-amino acids comprising culturing coryneform bacteria which includes an overexpressed sigE gene having a polynucleotide sequence which encodes the amino acid sequence of SEQ ID NO: 2 in a medium suitable for the expression of *sig*E to thereby produce L-amino acids. Accordingly, new claims 23 and 24 are directed to methods or processes using the unique nucleotide sequences of SEQ ID NO: 1 and the encoded amino acid sequence set forth in SEQ ID NO: 2 from *C. glutamicum* to produce L-amino acids, which is not disclosed in Kamura *et al.* New claims 25-28 ultimately depend from claim 24 (*e.g.*, vectors and host cells used in the claimed method) and therefore are also free of anticipation from Kamura *et al.* In view of the foregoing amendments and remarks, the applicants respectfully submit the rejection of claims 9, 12, 13, and 18 as being anticipated by Kamura *et al.* is moot, and should not be extended to new claims 23-28

CONCLUSION

In view of the foregoing, the claims are now believed to be in form for allowance, and such action is hereby solicited. If any point remains at issue which the examiner feels may be best resolved through a personal or telephone interview, the examiner is strongly urged to contact the undersigned at the number listed below.

Although the applicants believe no fee is necessary for consideration of this amendment/response, should the Patent Office determine otherwise, the Patent Office is authorized to charge such fee(s) to Deposit Acct. No. 033975 under Order No. 021123-0282664.

Respectfully submitted,
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